

DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE ORGANISM
§72-3(C) - SHRIMP

1. **CHEMICAL:** Novaluron PC Code No.: 124002
2. **TEST MATERIAL:** [Diffluorophenyl-¹⁴C(U)]Rimon Purity: >97%
3. **CITATION:**

Author: Machado, M.W.

Title: Novaluron - Acute Toxicity to Mysids (*Americamysis bahia*) Under Flow-Through Conditions

Study Completion Date: February 22, 2002

Laboratory: Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571-1075

Sponsor: Markhteshim Agan of North America, Inc.
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Laboratory Report ID: 11742.6142

MRID No.: 45638209

DP Barcode: D285479

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: *Rebecca Bryan*

Date: 4/1/03

APPROVED BY: Christie E. Padova, B.S., Staff Scientist, Dynamac Corporation

Signature: *Christie E. Padova*

Date: 4/1/03

5. **APPROVED BY:** Bill Evans, Biologist, OPP/EFED/ERB - I

Signature:

William Evans

Date: 11/20/03



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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Americamysis bahia*

Age or Size of Test Organism: <24 hours old

Definitive Test Duration: 96 hours

Study Method: Flow-through

Type of Concentration: Mean-measured

7. CONCLUSIONS:

The 96-hour acute toxicity of radiolabeled [difluorophenyl-¹⁴C(U)]Rimon (Novaluron) to the saltwater mysid, *Americamysis bahia*, was studied under flow-through conditions. Mysids were exposed to the test material at nominal concentrations of 0 (negative and solvent control), 0.032, 0.054, 0.090, 0.15, and 0.25 µg a.i./L; mean measured concentrations were <0.00039 (<LOQ; controls), 0.029, 0.045, 0.087, 0.18, and 0.25 µg a.i./L. At 96 hours, mortality was 0% in the control and 0.029 µg a.i./L levels, and 5, 5, 45, and 100% in the 0.045, 0.087, 0.18, and 0.25 µg a.i./L treatment levels, respectively. Lethargy and erratic swimming were observed in surviving mysids from the 0.18 µg/L treatment levels at 72 and 96 hours. The **96-hour LC₅₀ value (with 95% C.I.) was 0.13 (0.11 to 0.16) µg a.i./L**, which categorizes Rimon (Novaluron) as **very highly toxic** to the saltwater mysid, *Americamysis bahia*, on an acute toxicity basis. Based on mortality and sublethal effects, the **NOEC and LOEC values were 0.087 and 0.018 µg a.i./L**, respectively.

This study is scientifically valid and fulfills the requirements of an acute LC₅₀ test with an estuarine/marine organism (Subdivision E, §72-3(C) [shrimp]). This study is classified as **CORE**.

Results Synopsis**96-Hour:**

LC₅₀: 0.13 µg a.i./L 95% C.I.: 0.11-0.16 µg a.i./L

NOEC: 0.087 µg a.i./L

LOEC: 0.018 µg a.i./L

Endpoints affected: Mortality and sublethal effects

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: The guideline deviations were considered to be minor and did not impact the acceptability or validity of the study. Missing information should be provided to U.S. EPA.

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS:

1. The pretest mortality and signs of disease or injury of the mysid culture were not reported.
2. The water salinity ($20 \pm 3\text{‰}$) was less than recommended ($30\text{--}34\text{‰}$) for a marine (stenohaline) shrimp.
3. The water temperature ($24\text{--}25^{\circ}\text{C}$) was slightly higher than recommended ($22 \pm 1^{\circ}\text{C}$).
4. The test vessel size (1.6 L) and fill volume (1.4 L) were less than recommended.
5. The availability of [^{14}C]Rimon decreased significantly (up to 72%) between 0 and 96 hours.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of Novaluron to mysids for the purpose of chemical registration.

12. MATERIALS AND METHODS:**A. Test Organisms**

| Guideline Criteria | Reported Information |
|--|---|
| <u>Species</u> Preferred species are <i>Americamysis bahia</i> , <i>Penaeus setiferus</i> , <i>P. duorarum</i> , <i>P. aztecus</i> and <i>Palaemonetes</i> sp. | <i>Americamysis bahia</i> |
| <u>Age</u> Juvenile (≤ 24 hours old) mysids should be used | <24 hours old |
| <u>Supplier</u> | Juveniles were collected from in-house laboratory cultures. The original brood stock was obtained from Aquatic BioSystems, Inc., Ft. Collins, Colorado. |
| All shrimp are from same source? | Yes |
| All shrimp are from the same year class? | Not reported |

B. Source/Acclimation

| Guideline Criteria | Reported Information |
|---|----------------------|
| <u>Acclimation Period</u> Minimum 10 days | Continuous |
| Wild caught organisms were quarantined for 7 days? | N/A |
| Were there signs of disease or injury? | Not reported |
| If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing? | N/A |

| Guideline Criteria | Reported Information |
|--|--|
| <u>Feeding</u> No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study. | Fed live brine shrimp (<i>Artemia salina</i> nauplii) twice daily during acclimation and testing. |
| <u>Pretest Mortality</u> <3% mortality 48 hours prior to testing | Not reported |

C. Test System

| Guideline Criteria | Reported Information |
|--|---|
| <u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water | Artificial seawater prepared with laboratory dilution water and salt formula (hw-MARINEMIX®). |
| Does water support test animals without observable signs of stress? | Yes |
| <u>Salinity</u> 30-34 ‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range <6‰ | 20‰ |
| <u>Water Temperature</u> Approx. 22 ± 1 °C | 24-26°C |
| <u>pH</u> 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8 | 7.8-8.1 |
| <u>Dissolved Oxygen</u> Between 60 and 105% saturation. If needed, aerate prior to introduction of chemical. | 5.7-7.6 mg/L (77-103%) |

| Guideline Criteria | Reported Information |
|---|---|
| <u>Total Organic Carbon</u> Should be <5 mg/L in reconstituted seawater | 0.75 mg/L |
| <u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> 19.6 L is acceptable for organisms \geq 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. <u>Fill volume:</u> 15 L is acceptable for organisms \geq 0.5 g, 2-3 L is acceptable for smaller organisms. | 1. Glass battery jars, equipped with two drain holes covered with Nitex [®] 363 μ m screen 2. 1.6 L 3. 1.4 L |
| <u>Type of Dilution System</u> Must provide reproducible supply of toxicant | Intermittent-flow proportional diluter |
| <u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period | 7.2 aquarium volume additions/day; diluter systems were calibrated before and after the study and monitored for normal operation twice daily. |
| <u>Biomass Loading Rate</u> Static: \leq 0.8 g/L at \leq 17°C, \leq 0.5 g/L at $>$ 17°C; flow-through: \leq 1 g/L/day (N/A for mysids) | N/A |
| <u>Photoperiod</u> 16 hours light, 8 hours dark | 16 hours light, 8 hours dark, with a transition period. |
| <u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests | Acetone, 0.0049 mL/L |

D. Test Design

| Guideline Criteria | Reported Information |
|--|---|
| <p><u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 shrimp, then no definitive test is required.</p> | <p>Several studies were conducted prior to the final definitive study (pp. 17-18; refer to Reviewer's Comments section). In one preliminary study, ≤ 24 hour old mysid (10 mysids/level) were exposed via flow-through conditions to [difluorophenyl-$^{14}C(U)$Rimon at nominal concentrations of 0 (negative control), 0.065, 0.11, 0.18, 0.30, and 0.50 μg a.i./L. By 96 hours, there was 0, 10, 10, 90, 100, and 100% mortality in the control 0.065, 0.11, 0.18, 0.30, and 0.50 μg a.i./L treatment groups, respectively. The surviving mysid exposed at 0.18 μg a.i./L exhibited a complete loss of equilibrium.</p> |
| <p><u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.</p> | <p>0 (negative and solvent controls), 0.032, 0.054, 0.090, 0.15, and 0.25 μg a.i./L</p> |
| <p><u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers</p> | <p>20 mysids/level, divided into two replicates of 10 mysids each.</p> |
| <p><u>Test organisms randomly or impartially assigned to test vessels?</u></p> | <p>Yes</p> |
| <p><u>Biological observations made every 24 hours?</u></p> | <p>Yes</p> |

| Guideline Criteria | Reported Information |
|--|---|
| <u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control | 1. Measured daily in each aquarium and continuously in one solvent control vessel. 2. Measured daily in each aquarium. |
| <u>Chemical Analysis</u> needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used | Yes. LSC analysis was performed on samples collected from each test level at 0 and 96 hours, and HPLC/RAM analysis was performed on samples collected from the 0.25µg a.i./L (nominal) level at 0 and 96 hours. |

13. REPORTED RESULTS:**A. General Results**

| Guideline Criteria | Reported Information |
|--|--|
| Quality assurance and GLP compliance statements were included in the report? | Yes |
| <u>Recovery of Chemical</u> | <p>In a method validation study (November 2001) using unfiltered seawater and fortification levels of 0.00384, 0.0330, and 3.19 $\mu\text{g a.i./L}$, recoveries averaged $97.3 \pm 4.37\%$ with a LOQ of 0.000191 $\mu\text{g a.i./L}$ (Table 1A, p. 50).</p> <p>Based on QC samples prepared at each sampling interval at fortification levels of 0.0192, 0.0960, and 0.288 $\mu\text{g a.i./L}$ and analyzed (LSC) concurrently with the test samples, recoveries ranged from 93.9 to 106% of nominal (p. 15 and Table 2, p. 24).</p> |
| <u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior. | 0% mortality was observed in the negative and solvent controls. |
| Raw data included? | Yes |
| Signs of toxicity (if any) were described? | Yes |

Mortality

| Concentration ($\mu\text{g a.i./L}$) | | Number of Shrimp | Mean cumulative mortality (%) | | | |
|--|---------------|------------------|-------------------------------|----|-----|-----|
| Nominal | Mean Measured | | Hours of Study | | | |
| | | | 24 | 48 | 72 | 96 |
| Negative Control | <LOQ | 20 | 0 | 0 | 0 | 0 |
| Solvent Control | <LOQ | 20 | 0 | 0 | 0 | 0 |
| 0.032 | 0.029 | 20 | 0 | 0 | 0 | 0 |
| 0.054 | 0.045 | 20 | 0 | 0 | 0 | 5 |
| 0.090 | 0.087 | 20 | 0 | 0 | 5 | 5 |
| 0.15 | 0.18 | 20 | 0 | 40 | 45 | 45 |
| 0.25 | 0.25 | 20 | 5 | 85 | 100 | 100 |

Limit of quantitation = $0.00039 \mu\text{g a.i./L}$

At 96 hours, mortality was 0% in the control and $0.029 \mu\text{g a.i./L}$ levels, and 5, 5, 45, and 100% in the 0.045, 0.087, 0.18, and $0.25 \mu\text{g a.i./L}$ treatment levels, respectively. Lethargy and erratic swimming were observed in surviving mysids from the $0.18 \mu\text{g/L}$ treatment levels at 72 and 96 hours.

B. Statistical Results

Statistical Method: Using a computer program (Stephan, 1982), the 96-hour LC_{50} value (with 95% C.I.) was calculated using the moving average angle analysis. The 96-hour NOEC and LOEC were estimated by visual interpretation of the mortality and clinical observation data. Mean-measured concentrations were used in all estimations.

96-Hour:

LC_{50} : $0.13 \mu\text{g a.i./L}$

95% C.I.: $0.11\text{-}0.16 \mu\text{g a.i./L}$

NOEC: $0.029 \mu\text{g a.i./L}$

LOEC: $0.045 \mu\text{g a.i./L}$

Endpoints affected: Mortality and sublethal effects

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOEC and LOEC for mortality were determined using Fisher's Exact Test via TOXSTAT statistical software. The LC_{50} was determined using the moving average method via TOXANAL statistical software. Mean-measured concentrations were used in all estimations.

96-Hour:

LC_{50} : 0.13 $\mu\text{g a.i./L}$

95% C.I.: 0.11-0.16 $\mu\text{g a.i./L}$

NOEC: 0.087 $\mu\text{g a.i./L}$

LOEC: 0.18 $\mu\text{g a.i./L}$

Endpoints affected: Mortality and sublethal effects

15. REVIEWER'S COMMENTS:

The reviewer's conclusions for the NOEC and LOEC differed from those of the study author because of the different methods used to obtain these values. The reviewer's results are provided in the Executive Summary section. The reviewer's LC_{50} estimate was identical to the study author's.

Concentrations of available radioactivity, based on LSC analysis, declined significantly during the study, with decreases of 39-72% between sampling intervals (reviewer-calculated from data provided in Table 2, p. 24). At 0 hours, measured concentrations were 110-180% of nominal values and at 96 hours, measured concentrations were 44-68% of nominal. Based on the HPLC/RAM analysis of the highest test level, it was observed that Rimon did not undergo chemical degradation and accounted for 100% of the recovered radioactivity at 0 and 96 hours (Table 3, p. 25). Furthermore, the various measured diluter functions operated at 102% of their expected ranges throughout the experiment, and concurrently-run spiked QC samples ranged from 93.9 to 106% of nominal. An initial definitive study (described below) predicted the behavior of Rimon during the definitive study, and attempts were made to improve its concentration levels under test conditions by reducing the solvent concentration, utilizing artificial seawater, and employing a stricter test vessel cleaning regiment. The study author reported that the high measured concentrations at 0 hours are suspected to be due to collection of undissolved test substance or test substance which has sorbed to organic particles in solution, i.e., debris from brine shrimp, resulting in inflated measured concentrations (p. 19). Consequently, the lower values measured at 96 hours may be a result of cleaner exposure vessels where the undissolved test substance or sorbed particles may not be present. Since the decline of the test material was adequately addressed by the study author, this decline would alone not affect the acceptability of this study (refer to the Pesticide Reregistration Rejection Rate Analysis, Ecological Effects, Appendix A: Additional Supporting Documents, Conducting

Acceptable Aquatic Lab Studies: Proposed Guidance, pp. 3-9). This study satisfies guideline requirements and is classified as CORE.

Several preliminary experiments were conducted (p. 17). The first experiment was conducted under static conditions using ≤ 24 hour old mysids (2 replicates with five mysids per replicate) at nominal concentrations of 0 (control), 0.30, 1.5, and 3.0 $\mu\text{g a.i./L}$. After 96 hours, mortality was 0% in the control group, and 30, 100, and 100% in the 0.30, 1.5, and 3.0 $\mu\text{g a.i./L}$ groups, respectively. Two flow-through experiments were then conducted simultaneously in order to define concentrations for the definitive test and to evaluate the sensitivity of two age classes of mysids. One flow-through study was conducted with ≤ 24 hour old mysids (ten mysids per treatment) at nominal concentrations of 0 (control), 0.065, 0.11, 0.18, 0.30, and 0.50 $\mu\text{g a.i./L}$. After 96 hours, mortality was 0% in the control group, and 10, 10, 90, 100, and 100% in the 0.065, 0.11, 0.18, 0.30, and 0.50 $\mu\text{g a.i./L}$ groups, respectively. The surviving mysid from the 0.18 $\mu\text{g a.i./L}$ level exhibited a complete loss of equilibrium. The second flow-through study was with 5- to 6-day old mysid and otherwise as previously described. After 96 hours, mortality was 0% in the control and 0.065, and 0.11 $\mu\text{g a.i./L}$ groups, and 90, 100, and 100% in the 0.18, 0.30, and 0.50 $\mu\text{g a.i./L}$ groups, respectively. The surviving mysid from the 0.18 $\mu\text{g a.i./L}$ level exhibited darkened pigmentation and was lethargic.

Following the preliminary experiments, an initial definitive flow-through exposure was conducted with ≤ 24 -hour old mysids (10 per replicate, two replicates per level) at nominal concentrations of 0 (negative and solvent controls), 0.032, 0.054, 0.090, 0.15, and 0.25 $\mu\text{g a.i./L}$ (p. 18). The study was conducted in natural, filtered seawater (20‰) with a solvent (acetone) concentration of 100 $\mu\text{L/L}$. Respective mean-measured concentrations were 0.017, 0.029, 0.054, 0.089, and 0.16 $\mu\text{g a.i./L}$. After 96 hours, 0% mortality was observed in the control and 0.017 $\mu\text{g a.i./L}$ groups, and 10, 30, 40, and 100% mortality were observed in the 0.029, 0.054, 0.089, and 0.16 $\mu\text{g a.i./L}$ groups, respectively. Two mysids exposed at 0.054 $\mu\text{g a.i./L}$ exhibited darkened pigmentation, and all surviving mysids exposed at 0.089 $\mu\text{g a.i./L}$ were lethargic. The resultant LC_{50} (with 95% C.I.) was 0.076 (0.063-0.094) $\mu\text{g a.i./L}$. It was discovered in this study that concentrations of [^{14}C]novaluron declined significantly (actual results not provided) between the 0- and 96-hour sampling intervals, and resultant mean-measured values ranged from 52 to 63% of nominal concentrations. Since QC samples run concurrently with the test samples were consistent with the predetermined recovery ranges, it was concluded that solubility or adsorption was occurring in the seawater test system. At the request of the Study Sponsor, a second definitive study (described in this DER) was conducted in order to improve the variation in measured concentrations. The second exposure was conducted at a much lower solvent concentration (4.9 $\mu\text{L/L}$), utilized artificial seawater, and employed a stricter test vessel cleaning regiment.

HPLC/RAM characterization was only performed from samples collected at the highest test level of 0.25 $\mu\text{g a.i./L}$, and demonstrated [^{14}C]Rimon was stable under test conditions (HPLC recoveries correlated with LSC recoveries). Based on the HPLC analyses provided, Rimon accounted for 100% of the radioactive distribution (Table 3, p. 25).

This study conformed with Good Laboratory Practice Standards as published by the U.S. EPA GLP Regulations (40 CFR, Part 160) with the following exception: routine food and water contaminant screening analyses for pesticides, PCBs, and toxic metals were not collected in accordance with GLP procedures (p. 3). A Quality Assurance Statement was provided.

16. REFERENCES

- ASTM. 2000. Standard practice for conducting acute toxicity tests with fishes, microinvertebrates, and amphibians. Standard E729-96. American Society for Testing and Substances, Barr Harbor Drive, West Conshocken, PA. 19428.
- APHA, AWWA, WPCF. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edition, Washington, DC.
- Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. *Water Research*. 1:21-29.
- Reitsema, L.A. and J.M. Neff. 1980. A recirculating artificial seawater system for the laboratory culture of (Crustacea; Pericaridae). *Estuaries* 3: 321-323.
- U.S. EPA. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, D.C.
- U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-85-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.
- U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedure for Acute Toxicity Test for Estuarine and Marine Organisms. EPA-540/9-85-010. June 1985. U.S. Environmental Protection Agency, Washington, D.C.
- U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1035. Mysid Acute Toxicity Test. "Public Draft". EPA 712-C-96-136. April 1996. U.S. Environmental Protection Agency, Washington, D.C.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:
NOEC and LOEC

SUMMARY OF FISHERS EXACT TESTS

| GROUP | IDENTIFICATION | NUMBER EXPOSED | NUMBER DEAD | SIG (P=.05) |
|-------|----------------|-------------------|----------------|----------------|
| | CONTROL | 20 | 0 | |
| 1 | 0.029 | 20 | 0 | |
| 2 | 0.045 | 20 | 1 | |
| 3 | 0.087 | 20 | 1 | |
| 4 | 0.18 | 20 | 9 | * |
| 5 | 0.25 | 20 | 20 | * |

LC50

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|------|--------------|----------|------------------------------|----------|
| 4 | 4.215323E-02 | .1337218 | .1127149 | .1642764 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|------------|----------|----------|-----------------------------|
| 6 | 1.969894 | 5.262843 | 1.252711E-03 |

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.84827
 95 PERCENT CONFIDENCE LIMITS = -1.956418 AND 11.65296

LC50 = .1540699
 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 8.428854E-02
 95 PERCENT CONFIDENCE LIMITS = 0 AND .1603588